

Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations

Ronald Fayer^{*}, Monica Santin, James M. Trout

Environmental Microbial Safety Laboratory, Animal and Natural Resources Institute, Agricultural Research Service,
United States Department of Agriculture, Building 173, BARC-East, 10300 Baltimore Avenue,
Beltsville, Maryland 20705, USA

Received 16 August 2006; received in revised form 4 December 2006; accepted 7 December 2006

Abstract

Feces collected from 541 milking cows on two dairy farms each in Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida were examined for the presence of *Cryptosporidium* oocysts. Oocysts were concentrated from 15 g of feces from each cow and DNA was extracted. A two-step nested PCR protocol was used to amplify an 830 base pair fragment of the SSUrRNA gene. PCR-positive products were purified and sequenced. PCR-positive findings were obtained from cows in all seven states and from 11 of 14 farms. *Cryptosporidium parvum*, *Cryptosporidium bovis*, and *Cryptosporidium andersoni* were found on 2, 6, and 8 farms, and infected 0.4, 1.7, and 3.7% of the 541 cows, respectively. The overall lower prevalence of *Cryptosporidium* in these cows was very highly significant ($p \leq 0.0001$) compared with younger cattle and the relative prevalence of each species of *Cryptosporidium* also differed when compared with younger cattle previously examined on most of these same farms. The very low level of infection with *C. parvum*, the major species pathogenic to both cattle and humans, suggests that mature dairy cattle are a relatively low risk source of infection for humans.

Published by Elsevier B.V.

Keywords: Zoonoses; *Cryptosporidium parvum*; *Cryptosporidium bovis*; *Cryptosporidium andersoni*; Age; Molecular; Epidemiology

1. Introduction

In a series of four sequential annual investigations on nearly all the same dairy farms in seven states on the east coast of the United States, bovine feces were collected and examined to determine the prevalence and species of *Cryptosporidium* infecting commercial dairy cattle. During the first 2 years, calves from 5 days to 11 months of age were examined; 503 were pre-weaned

and 468 were post-weaned (Santín et al., 2004). During the third year, 571 dairy heifers 1–2 years of age were examined (Fayer et al., 2006). The overall prevalence of *Cryptosporidium* infecting these animals was high in pre-weaned calves and declined in older cattle. Within this time span changes were observed in the prevalence of three *Cryptosporidium* species and one genotype: *C. parvum*, *C. bovis*, *C. andersoni* and the *Cryptosporidium* deer-like genotype, respectively. The present study of 541 cows over 2 years of age encompasses the fourth year of this study and completes the life span of commercial dairy cattle from newborn calves to mature milking cows on these farms.

^{*} Corresponding author. Tel.: +1 301 504 8750;
fax: +1 301 504 6608.

E-mail address: rfayer@anri.barc.usda.gov (R. Fayer).

2. Materials and methods

2.1. Sources and collection of specimens

Feces were collected from 541 lactating Holstein cows more than 2 years of age on two dairy farms in each of the following states: Vermont, New York, Pennsylvania, Maryland, Virginia, North Carolina, and Florida (Table 1). All farms were visited once in 2005. The number of milking cows on each farm that provided feces for the present study ranged from 28 to 49 (Table 1). All 14 farms were the same as those visited in 2004 in a study of 12- to 24-month-old heifers (Fayer et al., 2006) and included 11 of the same farms visited in 2003 and 2002 in studies of post-weaned and pre-weaned calves (Santín et al., 2004).

2.2. Oocyst recovery, DNA extraction, and molecular characterization

All specimens were collected, transported, and processed as described (Santín et al., 2004). Oocysts were recovered from 15 g of feces from each cow and concentrated, washed, and pelleted as described (Santín et al., 2004). Likewise, DNA was extracted from the oocysts, a two-step nested PCR protocol was used to amplify an 830 bp fragment of the SSUrRNA gene, and PCR products were purified and sequenced using the same methods as described (Santín et al., 2004).

2.3. Statistical comparison

The prevalence of *Cryptosporidium* infection in these mature cows was compared with prevalence data for pre-weaned and post-weaned cattle from data previously collected on most of the same farms, except that farms PA-3, VA-3, NC-3, and NC-4 replaced farms where pre-weaned calves had been examined (Santín et al., 2004; Fayer et al., 2006) (Table 1). The χ^2 -test for independence was used to analyze the overall prevalence data and differences were considered very highly significant when $p \leq 0.0001$.

3. Results

3.1. Prevalence of *Cryptosporidium* by PCR and gene sequencing

Of the 31 PCR-positive specimens sequenced in the present study the following had 100% homology with genotypes listed in GenBank: 2 *C. parvum* (GenBank accession number: AF093490), 9 *C. bovis* (GenBank accession number: AY741305), and 20 *C. andersoni* (GenBank accession number: AB089285). Mixed infections were not detected.

PCR positive findings for the SSUrRNA gene of *Cryptosporidium* were obtained for 11 of 14 farms (Table 1). For one farm each in Vermont, Pennsylvania, and North Carolina *Cryptosporidium* was not detected

Table 1

The number (*n*) of cows from which a fecal specimen was collected and the number and prevalence of *Cryptosporidium* spp., *Cryptosporidium parvum*, *Cryptosporidium bovis*, and *Cryptosporidium andersoni* positive specimens from each farm

State	Farm	<i>n</i>	<i>Cryptosporidium</i>		<i>C. parvum</i>		<i>C. bovis</i>		<i>C. andersoni</i>	
			Positive	Prevalence	Positive	Prevalence	Positive	Prevalence	Positive	Prevalence
VT	VT-2	40	1	2.5	0	0	1	2.5	0	0
	VT-3	28	0	0	0	0	0	0	0	0
NY	NY-1	32	1	3.1	0	0	0	0	1	3.1
	NY-2	34	1	2.9	0	0	1	2.9	0	0
PA	PA-1	35	2	5.7	0	0	2	5.7	0	0
	PA-3	42	0	0	0	0	0	0	0	0
MD	MD-1	39	3	7.7	0	0	0	0	3	8
	MD-2	33	2	6.1	0	0	0	0	2	6
VA	VA-2	40	6	15.0	1	3	2	5.0	3	8
	VA-3	37	3	8.1	1	3	0	0	2	5.4
NC	NC-3	46	0	0	0	0	0	0	0	0
	NC-4	42	7	16.7	0	0	1	2	6	14.3
FL	FL-1	49	3	6.1	0	0	2	4.1	1	2
	FL-2	44	2	4.5	0	0	0	0	2	5
Total		541	31	5.7	2	0.4	9	1.7	20	3.7

in any of 28, 42, or 46 cows, respectively. The percentage of *Cryptosporidium*-positive cows ranged from 2.5% for a farm in Vermont (VT-2) to 16.7% for a farm in North Carolina (NC-4) (Table 1). *C. parvum*, *C. bovis*, and *C. andersoni* were found on 2, 6, and 8 of the 14 farms, respectively.

3.2. Prevalence of *Cryptosporidium*-positive cows related to age

Of 541 cows, 31 (5.7%) were found infected with *Cryptosporidium*. This overall prevalence of *Cryptosporidium* within the total population of cattle was lower in milking cows in the present study than in younger cattle on many of the same farms. The prevalence of infection decreased sequentially with the age of the animals, from pre-weaned calves to post-weaned calves, to heifers, and to milking cows. The prevalence of infection in each age group was 41, 26, 12, and 5.7%, respectively. These differences were very highly significant ($p \leq 0.0001$).

3.3. Prevalence of species related to age

Of the 541 cows, *C. parvum*, *C. bovis*, and *C. andersoni* constituted 0.4, 1.7, and 3.7%, respectively. Of the 31 *Cryptosporidium*-positive cows, *C. parvum*, *C. bovis*, and *C. andersoni* constituted 6, 29, and 65%, respectively (Fig. 1). Within that figure are data from the present study as well as data from previous studies

on most of the same farms. Each species and genotype of *Cryptosporidium* displayed a different prevalence pattern relative to the age of the cattle. An abrupt decrease was observed in the percentage of pre-weaned calves infected with *C. parvum* (85%) (Fig. 1A) versus the three older age groups infected with *C. parvum* (1–6%) (Fig. 1B–D). A steady increase in the percentage of *C. andersoni* was observed, from 1 to 13% in pre- to post-weaned calves, continuing to 44 and 65% in heifers and mature cows, respectively. The percentage of *C. bovis* infections increased abruptly from 9 to 55% in pre- to post-weaned calves, then decreased to 35 and 29% in heifers and mature cows, respectively. The percentage of infections with the *Cryptosporidium* deer-like genotype rose abruptly from 5 to 31% in pre- to post-weaned calves, and then declined to 13 and 0% in heifers and mature cows, respectively.

4. Discussion

Data from the present mature cows concludes the fourth year of an age group related study that provides documentation both of an overall reduction in the prevalence of *Cryptosporidium* in cattle of increasing age and of important changes in the species they harbor. In the present study, 31 (5.7%) of 541 cows were found infected. Within that group of cows, *C. andersoni*, *C. bovis*, and *C. parvum* were found in 65, 29, and 6% of the positive specimens. The present study is the first to find that *C. bovis* (Fayer et al., 2005) is present and

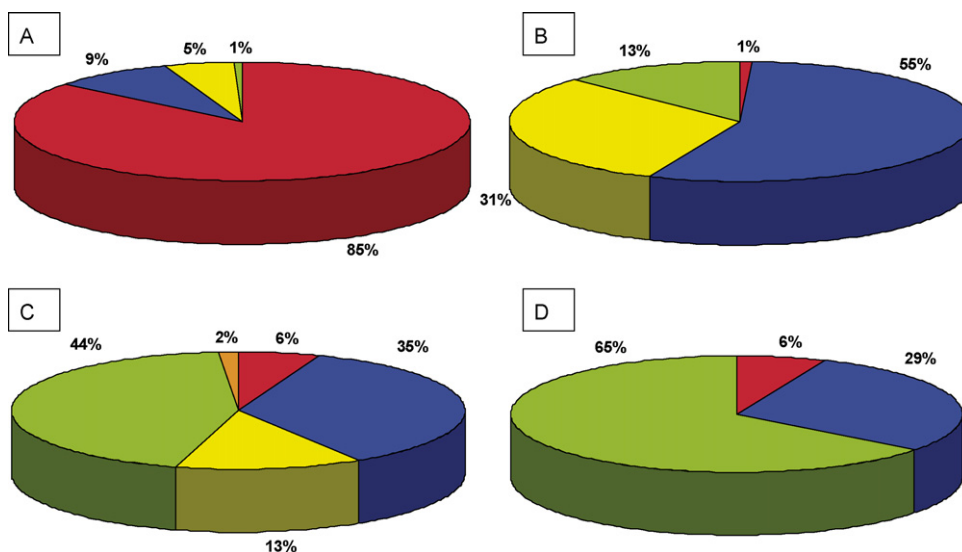


Fig. 1. Percentage of each species and genotype detected in *Cryptosporidium*-positive dairy cattle of different ages: (A) pre-weaned calves; (B) post-weaned calves, 3–11 months of age; (C) heifers, 12–24 months of age; (D) mature milking cows, >2 years of age. Red: *Cryptosporidium parvum*; blue: *Cryptosporidium bovis*; yellow: *Cryptosporidium* deer-like genotype; green: *Cryptosporidium andersoni*; orange: *Cryptosporidium suis*. Data for ages 1 week to 11 months of age were derived from previous publications (Santín et al., 2004; Fayer et al., 2006).

widespread in mature milking cows. The most likely explanation for this finding is that most published prevalence studies of *Cryptosporidium* in cattle have relied on microscopic observations incapable of identifying species and genotypes and therefore have identified *C. bovis* either as *Cryptosporidium* spp. or, erroneously based on the size of the oocysts, as *C. parvum*. Molecular methods have greatly helped to characterize *Cryptosporidium* oocyst isolates and reduce the confusion of taxonomy based on morphologically indistinguishable species and genotypes (Fayer et al., 2000; Xiao et al., 2004). Cows were not found to be infected with the *Cryptosporidium* deer-like genotype or with other species or genotypes.

The prevalence of *Cryptosporidium* species in lactating commercial dairy cows was determined on the basis of sequencing PCR positive products of the SSUrRNA gene of *Cryptosporidium*. The detection method used in the present study was identical to that used previously for detection of *Cryptosporidium* in younger dairy cattle (Santín et al., 2004; Fayer et al., 2006). This method had been tested for sensitivity by spiking 25 fecal specimens each with 10, 50, and 100 oocysts per gram and finding that 24, 56 and 84% were positive by PCR, respectively (Santín et al., 2004). Based on the sensitivity of the method, the present results most likely underestimate the actual number of infected cows, especially those excreting 10 or fewer oocysts per gram of feces. The actual prevalence is probably further underestimated when considering that only one fecal specimen was collected per cow and the possibility that that specimen could have been collected during a time of intermittent oocyst excretion.

In pre-weaned, post-weaned, and heifer calves on most of the same 14 dairy farms in seven eastern states *Cryptosporidium* was detected on 100, 100, and 92.9%

of the farms, respectively (Santín et al., 2004; Fayer et al., 2006). In the present study, *Cryptosporidium* was detected on 78.6% of those farms. In the most widespread geographic study in the United States, *Cryptosporidium* was detected on 59.2% of 1103 dairy farms in 28 states (Garber et al., 1993, 1994).

C. parvum, the only zoonotic species, and also the most pathogenic species for cattle, was most prevalent in pre-weaned calves, infecting 35% of all pre-weaned calves examined but diminishing in post-weaned calves, heifers, and mature cows to 0.2, 0.7 and 0.4%, respectively, of all animals examined (Santín et al., 2004; Fayer et al., 2006). *Cryptosporidium bovis* and the deer-like genotype found in these animals are host-specific for cattle and of no known health risk to humans or other animals. One heifer was infected with *C. suis* (Fayer et al., 2006), a rare infection in humans. *Cryptosporidium andersoni* was found in post-weaned calves, heifers, and cows. The only report of this species in humans identified 3 persons out of 2414 subjects with cryptosporidiosis in England between 1985 and 2000 (Leoni et al., 2006).

A comparison of all ages of dairy cattle, examined for *Cryptosporidium* by the same methods on nearly all of the same farms is presented in Fig. 2. The percentage of different species of *Cryptosporidium* detected in *Cryptosporidium* positive cattle of different ages is shown in Fig. 1. In pre-weaned calves, from 5 days of age to 2 months of age, 41% of 393 animals were infected with *Cryptosporidium*; *C. andersoni*, *C. bovis*, and *C. parvum* were found in 1, 9, and 85% of the positive specimens, respectively, and 5% were infected with the *Cryptosporidium* deer-like genotype (Santín et al., 2004). In post-weaned calves, 3–11 months of age, 26% of 447 animals were infected with *Cryptosporidium*; *C. andersoni*, *C. bovis*, and *C. parvum* were

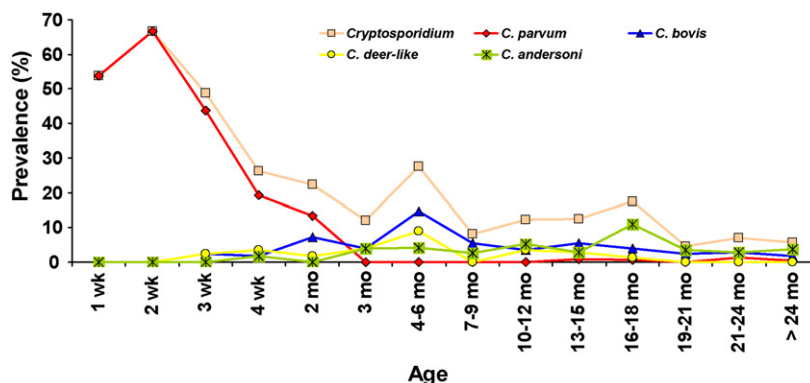


Fig. 2. Prevalence of infected cattle from 1 week of age to >2 years of age by species and genotype derived from all animals examined on 14 farms in seven states over a period of 4 years. Data for ages 1 week to 11 months of age were derived from previous publications (Santín et al., 2004; Fayer et al., 2006).

found in 13, 55, and 1% of the positive specimens, respectively, and 31% were infected with the *Cryptosporidium* deer-like genotype (Santín et al., 2004). In 12- to 24-month-old heifers, 12% of 571 animals were infected with *Cryptosporidium* and whereas *C. andersoni*, *C. bovis*, and *C. parvum* were found in 44, 35, and 6% of the positive specimens, 13% were infected with the *Cryptosporidium* deer-like genotype, and 2% with *C. suis* (Fayer et al., 2006).

Similar findings with regard to overall prevalence of cryptosporidiosis in dairy cattle of different ages have been reported worldwide based primarily on microscopy alone. Although species and genotypes could not be determined by microscopy, trends are discernable. In central, northern, southeastern and western United States, <2.5% of dairy calves <4 days old tested positive for cryptosporidiosis (Garber et al., 1993, 1994). The rate increased to 48% of dairy calves 1–3 weeks of age, decreased to 21.9% for 3- to 5-week-old calves and was <15% for calves older than 5 weeks of age (Garber et al., 1993, 1994). In Ontario, Canada 40.6% of 500 7- to 21-day-old calves on 51 farms were infected with *C. parvum* (Trotz-Williams et al., 2005). In central Mexico 25% of 512 1- to 30-day-old calves on 31 dairy farms were determined to be infected with *C. parvum* based on carbol fuchsin stained fecal smears (Maldonado-Camargo et al., 1998). In central Spain feces were examined from 218 1- to 30-day-old diarrheic calves on 65 dairy farms (de la Fuente et al., 1999). *Cryptosporidium* infection was detected in calves at 1–7, 8–14, 15–21 and 22–30 days of age at the rate of 43.8, 71.9, 63.2 and 6.9%, respectively (de la Fuente et al., 1999). In Galicia (NW Spain) 47.9% of 844 calves less than 1 month of age on 22 farms were found infected with *Cryptosporidium* by microscopy (Castro-Hermida et al., 2002). In Israel 100% of 145 acid-fast stained fecal smears from 7- to 13-day-old calves, were found positive for *C. parvum* (Tanriverdi et al., 2006). In the Czech Republic 56.5% of 2-week-old calves on 11 dairy farms were found to be infected with *C. parvum* whereas no calves over 2 months of age were found to be infected with *Cryptosporidium*, based on immunofluorescence microscopy (Kvac et al., 2006). In France, two surveys of dairy calves less than 3 weeks of age detected *Cryptosporidium* in 18% of 1680 calves and 43% of 440 calves by ELISA (Lefay et al., 2000). In Zambia 42.8% of 250 fecal specimens from calves up to 3 months of age on 37 dairy farms were found infected with *Cryptosporidium* by microscopy (Guerden et al., 2006). Genotyping of 32 positive specimens revealed 68.8% to be *C. parvum* and 31.2% to be *C. bovis* (Guerden et al., 2006). In Denmark, 1 g of feces from 10

calves less than 1 month of age, 10 calves 1–12 months of age, and five milking cows from each of 50 dairy farms were examined by immunofluorescence methods (Maddox-Hyttel et al., 2006). The age specific prevalence of *Cryptosporidium* was 31, 100, and 16%, respectively. Species were not identified. Many other studies have provided prevalence data for *Cryptosporidium* in dairy cattle but have not identified the species or age of the infected animals (Castro-Hermida et al., 2002).

Somewhat lower prevalence data have been reported in other studies of dairy cattle. In New York State in eastern United States 2943 fecal specimens were examined by brightfield and phase-contrast microscopy from cattle on 109 dairy farms (Wade et al., 2000). The prevalence of infection with *C. parvum* and *C. andersoni* was found to be 0.9 and 1.1%, respectively, much lower than in other reported studies. Cattle were also studied at three stratified age groups: <6 months of age, 6–24 months of age, and >24 months of age. In these age groups the prevalence of *C. parvum* was 2.4, 0, and 0%, and the prevalence of *C. andersoni* was 0.5, 1.7, and 1.5%, respectively. These low values possibly reflect the detection method used for oocysts but show a decreased prevalence of *C. parvum*-sized oocysts with increasing age and an increase in prevalence of *C. andersoni*-sized oocysts. In a second study of dairy calves in the New York watershed by this group 7.5% of 9914 fecal specimens collected from 6530 calves were found positive for *Cryptosporidium* by ELISA (Starkey et al., 2005). Although the study was conducted over a period of 2 years, all positive specimens were found in calves less than 61 days of age and most were found in calves 7–21 days of age. Also in northeastern United States, feces were examined from 557 calves 0–3, 4–8, and 9–12 weeks of age and from 127 mature cows on 11 dairy farms (Sischo et al., 2000). Oocysts identified by immunofluorescence microscopy were found in 15, 2, and 8% of calves in the three age groups, respectively, and in 2% of the mature cows. Again, a similar trend in prevalence versus age was observed. In Norway immunofluorescence microscopy of 3 g of feces from 1386 calves on 136 farms revealed a relatively low prevalence of infection in 3 to 183-day-old dairy calves (Hamnes et al., 2006). Data were obtained for six age groups. The prevalence of oocysts in calves 3–31 and 154–183 days of age was only 7%. The remaining four age groups bracketed within these two had prevalences of 11–17%. No oocysts had the appearance of *C. andersoni*.

Oocysts of *C. andersoni*, resemble those of *C. muris* in size, but were found not to infect rodents (Lindsay

et al., 2000). The first surveys to determine the prevalence of *C. andersoni* (referred to as *C. muris* in earlier reports) were conducted by Bruce Anderson. In California, of 8539 fecal specimens from mature dairy cows and heifers (post-weaning to near term gestation), 1.7% were found infected with *C. andersoni* (Anderson, 1990). Detection was based on observation of acid-fast stained microscope slides, each containing 90 4–5 mm fecal dots, each dot representing feces transferred from the end of a wood applicator stick thrust into the moist center of a manure deposit. Conceivably such a small quantity of feces would underestimate the actual number of positive animals. However, similar findings have been reported by others. In Spain, 1.5% of 131 asymptomatic mature cattle were considered to be infected with *C. muris* based on the size of carbol fuchsin stained oocysts (Lorenzo Lorenzo et al., 1993). In Japan, 4.7% of 514 fecal specimens collected from dairy cattle at an abattoir were considered to be infected with *C. muris* (Kaneta and Nakai, 1998). The foregoing are similar to the findings in the present study in which the prevalence of *C. andersoni* was found to be 3.7% of 541 mature dairy cows. The highest prevalence of *C. andersoni*, albeit with a similar age related trend, was reported from the Czech Republic (Kvac et al., 2006). The prevalence increased steadily beginning with only one 29-day-old dairy calf to over 35% of 5-month-old calves and thereafter (only a single herd was examined) to 100% of 11-month-old calves.

In the Netherlands, based on immunofluorescence microscopy, peak periods of oocyst excretion were observed in dairy calves during the first month of age and at 6 and 12 months of age (Huetink et al., 2002). A similar pattern is present in Fig. 2, wherein the first peak in prevalence was found in the first month of age, the second peak at 4–6 months of age and the third peak 16–18 months of age. Each of the three peaks are represented in part by different species or genotypes: *C. parvum*, then *C. bovis* and *Cryptosporidium* deer-like genotype, and then *C. bovis* and *C. andersoni*, respectively (Fig. 1). The especially high peak prevalence observed in the first month age, due primarily to *C. parvum*, possibly reflects two factors: the immature immune status of young cattle (Fayer et al., 1998) and the highly infectious nature of this species. The use of molecular methods to identify the organisms present during these peak times provides a valid basis for risk assessment. The major concern for public health and for bovine health is the presence of *C. parvum*. Based on the present findings as well as those of younger dairy cattle from most of the same farms (Santín et al., 2004; Fayer et al., 2006), as well as

generalized prevalence data from dairy cattle worldwide, it appears that pre-weaned calves, mostly those less than 2 months of age, are the primary source of this species and present the greatest risk for transmission to humans.

Acknowledgements

The authors thank Kristin Cameron and Brooke Reich for technical services in support of this study.

References

- Anderson, B.C., 1990. A preliminary report on prevalence of *Cryptosporidium muris* oocysts in dairy cattle feces. California Vet. 11–12.
- Castro-Hermida, J.A., Gonzalez-Losada, A., Ares-Mazas, E., 2002. Prevalence of and risk factors involved in the spread neonatal bovine cryptosporidiosis in Galicia (NW Spain). Vet. Parasitol. 106, 1–10.
- de la Fuente, R., Luzon, M., Ruiz-Santa-Quiteria, J.A., Garcia, A., Cid, D., Orden, J.A., Garcia, S., Sanz, R., Gomez-Bautista, M., 1999. *Cryptosporidium* and concurrent infections with other major enteropathogens in 1- to 30-day-old diarrheic dairy calves. Vet. Parasitol. 80, 179–185.
- Fayer, R., Gasbarre, L., Pasquali, P., Canals, A., Almeria, S., Zarlenga, D., 1998. *Cryptosporidium parvum* infection in bovine neonates: dynamic clinical parasitic and immunologic patterns. Int. J. Parasitol. 28, 49–56.
- Fayer, R., Morgan, U., Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 30, 1305–1322.
- Fayer, R., Santín, M., Xiao, L., 2005. *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) from cattle (*Bos taurus*). J. Parasitol. 91, 624–629.
- Fayer, R., Santín, M., Trout, J.M., Greiner, E., 2006. Prevalence of species and genotypes of *Cryptosporidium* found in 1- to 2-year-old dairy cattle in eastern United States. Vet. Parasitol. 135, 105–112.
- Garber, L.P., Salman, M.D., Hurd, H.S., 1993. *Cryptosporidium* in dairy calves: a National Animal Health Monitoring System study. In: Proceedings of the Livestock Conserv. Inst., April 14–16, 1993, pp. 42–47.
- Garber, L.P., Salman, M.D., Hurd, H.S., Keefe, T., Schlater, J.L., 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. 205, 86–91.
- Guerden, T., Goma, F.Y., Siwila, J., Phiri, I.G.K., Mwanza, A.M., Gabriel, S., Claerebout, Vercruysse, J., 2006. Prevalence and genotyping of *Cryptosporidium* in three cattle husbandry systems in Zambia. Vet. Parasitol. 138, 217–222.
- Hamnes, I.S., Gjerde, B., Robertson, L., 2006. Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol. 140, 204–216.
- Huetink, R.E.C., van der Giessen, J.W.B., Noordhuizen, J.P.T.M., Ploeger, H.W., 2002. Epidemiology of *Cryptosporidium parvum* and *Giardia duodenalis* on a dairy farm. In: Food Safety Assurance in the Pre-Harvest Phase, vol. 1: Food Safety Assurance and Veterinary Public Health, pp. 262–264.
- Kaneta, Y., Nakai, Y., 1998. Survey of *Cryptosporidium* oocysts from mature cattle in a slaughter house. J. Vet. Med. Sci. 60, 585–588.

- Kvac, M., Kouba, M., Vitovec, J., 2006. Age-related and housing-dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Vet. Parasitol.* 137, 202–209.
- Lefay, D., Naciri, M., Poirier, P., Chermette, R., 2000. Prevalence of *Cryptosporidium* infection in calves in France. *Vet. Parasitol.* 89, 1–9.
- Leoni, F., Amar, C., Nichols, G., Pedraza-Diaz, S., McLauchlin, J., 2006. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. *J. Med. Microbiol.* 55, 703–707.
- Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R., Blagburn, B.L., 2000. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle, *Bos taurus*. *J. Eukaryot. Microbiol.* 47, 91–95.
- Lorenzo Lorenzo, M.J., Ares-Mazas, E., Villacorta, I., 1993. Detection of oocysts and IgG antibodies to *Cryptosporidium parvum* in asymptomatic mature cattle. *Vet. Parasitol.* 47, 9–15.
- Maddox-Hyttel, C., Langkjaer, R.K., Enemark, H.L., Vigre, H., 2006. *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs—occurrence and management associated risk factors. *Vet. Parasitol.* 141, 48–59.
- Maldonado-Camargo, S., Atwill, E.R., Saltijeral-Oaxaca, J.A., Herrera-Alonso, L.C., 1998. Prevalence of and risk factors for shedding of *Cryptosporidium parvum* in Holstein–Freisian dairy calves in central Mexico. *Prevent. Vet. Med.* 36, 95–107.
- Santín, M., Trout, J.M., Xiao, L., Zhou, L., Greiner, E., Fayer, R., 2004. Prevalence and age related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet. Parasitol.* 122, 103–117.
- Sischo, W.M., Atwill, E.R., Lanyon, L.E., George, J., 2000. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. *Prevent. Vet. Med.* 43, 253–267.
- Starkey, S.S., Wade, S.E., Schaaf, S., Mohammed, H.O., 2005. Incidence of *Cryptosporidium parvum* in the dairy cattle population in a New York City watershed. *Vet. Parasitol.* 131, 197–205.
- Tanriverdi, S., Markovics, A., Arslan, M.O., Itik, A., Shklap, V., Widmer, G., 2006. Emergence of distinct genotypes of *Cryptosporidium parvum* in structured host populations. *Appl. Environ. Microbiol.* 72, 2507–2513.
- Trotz-Williams, L.A., Jarvie, B.D., Martin, S.W., Leslie, K.E., Peregrine, A.S., 2005. Prevalence of *Cryptosporidium parvum* infection in southwestern Ontario and its association with diarrhea in neonatal dairy calves. *Can. Vet. J.* 46, 349–351.
- Wade, S.E., Mohammed, H.O., Schaaf, S.L., 2000. Prevalence of *Giardia* sp. and *Cryptosporidium parvum* and *Cryptosporidium muris* (*C. andersoni*) in 109 dairy herds in five counties in southeastern New York. *Vet. Parasitol.* 93, 1–11.
- Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin. Microbiol. Rev.* 17, 72–97.